PATENT SPECIFICATION (11) 1357 121

1357 121

(21) Application No. 11048/71 (22) Filed 23 April 1971

(31) Convention Application No. 58686 (32) Filed 28 July 1970 in

(33) United States of America (US)

(44) Complete Specification published 19 June 1974

(51) International Classification C07C 103/52

(52) Index at acceptance

C2C 1341 1343 1410 213 215 246 247 250 251 252 25Y 28X 30Y 320 321 32Y 332 340 342 34Y 351 352 355 360 361 366 367 36Y 575 576 586 58Y 596 604 620 627 62X 630 635 637 63X 645 64X 678 70Y 738 790 79Y KB KN KW KZ

(19)

(54) PEPTIDE ENZYME INHIBITORS

(71) We, E. R. SQUIBB & SONS INC. a Corporation organised under the laws of the State of Delaware, United States of America, of 909 Third Avenue, New York, New York 10022, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us and the method by which it is to be performed to be particularly described in and by the following statement: —

This invention relates to peptide enzyme inhibitors.

The action of the enzyme renin or renin substrate, a pseudoglobulin in blood plasma, produces a polypeptide angiotensin I, also known as hypertensin I. The latter is converted by an enzyme to angiotensin II, also known as hypertensin II or angiotonin. Angiotensin II is an active pressor substance which is present in the plasma of warm blooded animals with essential hypertension in quantities sufficient to maintain elevated blood pressure. Inhibition of the enzyme responsible for the conversion of angiotensin I to angiotensin II serves to remove a cause of essential hypertension.

It is, accordingly, an objection of the present invention to provide a series of new peptides which, as such, or in some cases in the form of protected derivatives, inhibit the conversion of angiotensin I into angiotensin II and which are effective in relieving essential hypertension. A further object is to provide a method for alleviating essential hypertension in non-humans. These and other objects of the present invention will be apparent from the following description.

It has now been found that the enzymatic conversion of angiotensin I into angiotensin II is inhibited by certain peptides or protected derivatives thereof of the following classes:

1. Nona- or decapeptides in which the amino acid sequence

$$Pyr\text{-}(X)_n\text{-}Trp\text{-}Pro\text{-}Y\text{-}Pro\text{-}Z\text{-}Ile\text{-}Pro\text{-}Pro$$

wherein X is an asparagine, serine, or norleucine moiety, n is 0 or 1, Y is an arginine, histidine, lysine or glycine moiety, and Z is an asparagine or glutamine moiety;

II. Penta- or hexapeptides in which that sequence is

$$Pyr\text{-}(X)_n\text{-}Trp\text{-}Pro\text{-}Y\text{-}Pro$$

where n, X and Y are as defined in I; and

III. tetrapeptides in which that sequence is

$$Trp\text{-}Pro\text{-}Y\text{-}Pro$$

where Y is as defined in I, and in which the terminal amino group is protected.

The first category, I, of the peptides of the present invention are nona- or decapeptides having the amino acid sequence:

$$Pyr\text{-}(X)_n\text{-}Trp\text{-}Pro\text{-}Y\text{-}Pro\text{-}Z\text{-}Ile\text{-}Pro\text{-}Pro$$

wherein X, n, Y and Z are as defined above and protected derivatives thereof. Specific compounds include in this category are the following:

1. Pyr-Asn-Trp-Pro-Arg-Pro-Ash-Ile-Pro-Pro
2. Pyr-Ser-Trp-Pro-Arg-Pro-Asn-Ile-Pro-Pro
3. Pyr-Nle-Trp-Pro-Arg-Pro-Asn-Ile-Pro-Pro
4. Pyr-Trp-Pro-Arg-Pro-Asn-Ile-Pro-Pro
5. Pyr-Asn-Trp-Pro-His-Pro-Asn-Ile-Pro-Pro
6. Pyr-Ser-Trp-Pro-His-Pro-Asn-Ile-Pro-Pro
7. Pyr-Nle-Trp-Pro-His-Pro-Asn-Ile-Pro-Pro
8. Pyr-Trp-Pro-His-Pro-Asn-Ile-Pro-Pro
9. Pyr-Asn-Trp-Pro-Lys-Pro-Asn-Ile-Pro-Pro
10. Pyr-Ser-Trp-Pro-Lys-Pro-Asn-Ile-Pro-Pro
11. Pyr-Nle-Trp-Pro-Lys-Pro-Asn-Ile-Pro-Pro
12. Pyr-Trp-Pro-Lys-Pro-Asn-Ile-Pro-Pro-

[Price 25p]

13. Pyr-Asn-Trp-Pro-Gly-Pro-Asn-Ile-Pro Pro
14. Pyr-Ser-Trp-Pro-Gly-Pro-Asn-Ile-Pro-Pro
15. Pyr-Nle-Trp-Pro-Gly-Pro-Asn-Ile-Pro-Pro
16. Pyr-Trp-Pro-Gly-Pro-Asn-Ile-Pro-Pro
17. Pyr-Asn-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro
18. Pyr-Ser-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro
19. Pyr-Nle-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro
20. Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro
21. Pyr-Asn-Trp-Pro-His-Pro-Gln-Ile-Pro-Pro
22. Pyr-Ser-Trp-Pro-His-Pro-Gln-Ile-Pro-Pro
23. Pyr-Nle-Trp-Pro-His-Pro-Gln-Ile-Pro-Pro
24. Pyr-Trp-Pro-His-Pro-Gln-Ile-Pro-Pro-
25. Pyr-Asn-Trp-Pro-Lys-Pro-Gln-Ile-Pro-Pro
26. Pyr-Ser-Trp-Pro-Lys-Pro-Gln-Ile-Pro-Pro
27. Pyr-Nln-Trp-Pro-Lys-Pro-Gln-Ile-Pro-Pro
28. Pyr-Trp-Pro-Lys-Pro-Gln-Ile-Pro-Pro
29. Pyr-Tsn-Trp-Pro-Gly-Pro-Gln-Ile-Pro-Pro
30. Pyr-Ser-Trp-Pro-Gly-Pro-Gln-Ile-Pro-Pro
31. Pyr-Nle-Trp-Pro-Gly-Pro-Gln-Ile-Pro-Pro
32. Pyr-Trp-Pro-Gly-Pro-Gln-Ile-Pro-Pro

The second category, II, of the peptides of the present invention are penta- or hexapeptides having the amino acid sequence:

$$Pyr\text{-}(X)_n\text{-}Trp\text{-}Pro\text{-}Y\text{-}Pro$$

where X, n and Y are as defined for category I. Specific compounds include in this category are the following:

33. Pyr-Asn-Trp-Pro-Arg-Pro
34. Pyr-Ser-Trp-Pro-Arg-Pro
35. Pyr-Nle-Trp-Pro-Arg-Pro
36. Pyr-Trp-Pro-Arg-Pro
37. Pyr-Asn-Trp-Pro-His-Pro
38. Pyr-Ser-Trp-Pro-His-Pro
39. Pyr-Nle-Trp-Pro-His-Pro
40. Pyr-Trp-Pro-His-Pro
41. Pyr-Asn-Trp-Pro-Lys-Pro
42. Pyr-Ser-Trp-Pro-Lys-Pro
43. Pyr-Nle-Trp-Pro-Lys-Pro
44. Pyr-Trp-Pro-Lys-Pro
45. Pry-Asn-Trp-Pro-Gly-Pro
46. Pyr-Ser-Trp-Pro-Gly-Pro
47. Pyr-Nle-Trp-Pro-Gly-Pro
48. Pyr-Trp-Pro-Gly-Pro

The third Category, III, of the peptides of the present invention are tetrapeptides having the amino-acid sequence

$$Trp\text{-}Pro\text{-}Y\text{-}Pro$$

where Y is as defined for the first category I and derivatives in which at least the amino group of the tryptophyl radical is protected. Specific protected compounds include in this category are the following:

49 R-Pro-Arg-Pro
50 R-Pro-His-Pro
51. R-Pro-Lys-Pro
52. R-Pro-Gly-Pro

where R is a tryptophyl radical in which the amino group is protected.

The compounds of the first and second categories may be used as the free, i.e. unprotected peptide, or as an N-protected peptide or a C-protected peptide, or both. The compounds of the third category are used in the form of the N-protected tetrapeptides. The C-terminal group may also be protected. In the compounds of all three categories, other functional groups, e.g. hydroxyl and guanidino, as well as amino groups other than the N-terminal amino and carboxyl groups other than the C-terminal carboxyl, may also be protected.

The choice of carboxyl protecting group will depend on various factors, e.g., the nature of the peptide being synthesized, ease of removal of protecting group, reaction solvent and temperature.

Some commonly used methods of protecting carboxyl groups are the by converting them into:

1. salts
2. lower alkyl ester groups
3. phenyl substituted lower alkyl ester groups, e.g., benzyl and benzhydryl ester groups
4. p-nitrobenzyl ester groups
5. p-methoxybenzyl ester groups
6. phthalimidomethyl ester groups
7. tertiarybutyl ester groups
8. cyclopentyl ester groups
9. methylthioethyl ester groups
10. trimethylsilyl groups
11. hydrazide groups

Some specific carboxyl protecting groups are methyl, ethyl, propyl, tert.-butyl, and benzyl. A more complete listing may be obtained by reference to standard works on peptide synthesis, e.g. Bodanszky et al., "Peptide Synthesis", chapter 4, Interscience publishers, 1966, or Schroder et al., "The Peptides", Vol. I, pp. xxiii—xxix, Academic Press, 1965.

The N-terminal group of the peptides of the present invention may be protected group in accordance with the skill of the art. The choice of amino protecting group will depend on various factors, e.g., the nature of the amino acid or peptide which is to be attached to the N-terminal group of the amino acid or peptide, the ease of removal of the protecting group, reaction solvent and temperature. Some common examples of amino protecting and amino protected groups are:

1. amine hydrochlorides
2. the p-toluenesulfonyl group
3. the benzoyloxycarbonyl (carbobenzoxy) group
4. substituted benzyloxycarbonyl and other urethane protecting groups
5. the trifluoroacetyl group
6. the phthalyl (or phthaloyl) group
7. the diphenylmethyl (benzhydryl) and triphenylmethyl (trityl) groups
8. the formyl group
9. lactam groups
10. Schiff bases and enamines
11. the benzylsulfonyl group
12. tritylsulfonyl and arylsulfonyl groups.

Some specific amino-protecting groups are tert. - butyloxycarbonyl, o - nitrophenylsulfonyl, and tosyl. A more complete listing may be obtained by reference to standard works on peptide synthesis, e.g., Bodanszky et al., supra, or Schroder et al., supra.

Examples of suitable hydroxyl protecting groups are, for example, benzyl, t-butyl, and tetrahydropyranyl. Examples of suitable guanidine protecting group are, nitro, tosyl, p-nitrobenzyloxycarbonyl, and adamantyloxycarbonyl groups, and protection by protonation may also be used. A more complete listing of hydroxyl and guanidine protecting groups may be had by reference to standard works on peptide, synthesis, for example, the previously mentioned Bodansky et al. or Schroder et al. texts.

The compounds of the present invention may be used per se or in the form of their physiologically - acceptable peptide salts. Examples of such salts are acid-addition salts, such as, for example, hydrochloride, hydrobromide, acetate, and haloacetate such as trifluoroacetate and dichloroacetate.

The compounds of the present invention inhibit the conversion of angiotensin I into angiotensin II. At a concentration of from about 0.05 to about 10 mcg/ml they inhibit conversion of 50% angiotensin I, the latter having a concentration of 5 mM. At a dosage level of from about 0.5 to about 5 mg/kg, the compounds of the present invention are effective in reducing hypertension in the rat. For this purpose they may be administered orally or parenterally by incorporating the appropriate dosage of a compound according to the present invention with a physiologically-acceptable carrier.

The compounds of the present invention are useful as biodegradable ultraviolet absorbents and as such may be used in suntan-sunscreen products.

The preparation of the peptides of the present invention of the formula

R^1-Pro-Y-Pro-B

wherein Y is an arginine, histidine, lysine or glycine moiety, B is —OH or

—X-Ile-Pro-Pro

wherein Z is an asparagine or glutamine moiety, and R^1 is a tryptophyl radical in which the amino group is protected of

Pyr(X)$_n$-Trp

when B is —OH or Pyr(X)$_n$-Trp when B is —Z-Ile-Pro-Pro, X is an asparagine, serine or norleucine moiety and n is zero or 1, will now be described.

The peptides are prepared in the conventional manner, building up the chain by introducing groups utilizing reactions well known in the art and illustrated in the examples following.

Specifically, examples 1 through 6 show the preparation of the chain Z-Ile-Pro-Pro (see parts 1 through 12 of example 1); and also the addition thereto and the preparation of the central portion of the chain, i.e.,

Trp-Pro-Y-Pro

(see steps 16 to 21 of example 1 and examples 12 through 16). The foregoing results in the preparation of the peptide chain

Trp-Pro-Y-Pro-B

where B is —OH or —Z-Ile-Pro-Pro, Z and Y being as previously defined.

These intermediate peptides have the Trp-radical converted to the R^1 group by conventional reaction methods illustrated by steps 22 through 28 of example 1, the amino group of said radical being protected in the case where B is —OH; the group X being added where n is one in the generic formula, and finally, the pyroglutaminyl group being added.

The following examples illustrate the invention. All amino acids are of the L-configuration.

Example 1

Pyroglutamyl - asparaginyl - tryptophyl - prolyl - histidyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

Butyloxycarbonylprolyl resin (8 g. containing ca. 0.5 meq. of proline per gram) is allowed to stir overnight with dichloromethane. The dichloromethane is removed by filtration and the resin is submitted to the following cycles of manipulations:

1. Wash four times each with dichloromethane, absolute ethanol and acetic acid.

2. Shake for 5 minutes with 80 ml of 1N HCl in acetic acid, repeating the same procedure for another 25 minutes with fresh 1N HCl in acetic acid.

3. Wash four times each with acetic acid, absolute ethanol and dichloromethane.

4. Shake for 5 minutes with a 10% solution of triethylamine in chloroform (28 ml). Repeat this procedure once.

5. Wash four times each with chloroform and dichloromethane.

6. Shake with a solution of t - butyloxycarbonylproline (2.57 g) in dichloromethane (56 ml) for 20 minutes.

7. Add a solution of dicyclohexylcarbodi-

imide (2.5 g) in dichloromethane (4 ml) and continue the shaking for 3 hours.

8. Repeat steps 1, 2, 3, 4 and 5.

9. Add a solution of t-butyloxycarbonyl-isoleucine (2.9 g) in dichloromethane (56 ml) and shake the suspension for 20 minutes.

10. Repeat steps 7, 1, 2, 3, 4 and 5.

11. Add a solution of t-butyloxycarbonyl-glutamine p - nitrophenyl ester (2.2 g) in a mixture of dimethylformamide (8 ml) and dichloromethane (20 ml) and shake the suspension overnight.

12. Wash four times with dichloromethane and add a solution of t - butyloxycarbonyl - glutamine p - nitrophenyl ester (1.46 g) in a mixture of dimethylformamide (8 ml) and dichloromethane (20 ml) and shake for 5 hours.

13. Repeat steps 1, 2, 3, 4 and 5.

14. Repeat coupling step 6 and 7.

15. Repeat steps 1, 2, 3, 4 and 5.

16. Add a solution of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl histidine (4.6 g) in dichloromethane (45 ml) and shake for 20 minutes.

17. Repeat steps 7, 1, 2, 3, 4 and 5.

18. Repeat coupling steps 6 and 7.

19. Repeat steps 1, 2, 3, 4 and 5.

20. Add a solution of 5 - butyloxycarbonyl - tryptophan (3.67 g) in a mixture of dimethylformamide (15 ml) and dichloromethane (41 ml) and shake for 20 minutes.

21. Repeat steps 7, 1, 2, 3, 4 and 5, but adding 1% of mercaptoethanol and 18% of anisole in all acidic washings.

22. Add a solution of t-butyloxycarbonyl-asparagine p - nitrophenyl ester (3.54 g) in a mixture of dimethylformamide (15 ml) and dichloromethane (41 ml) and shake overnight.

23. Wash four times with dichloromethane, add a solution of t - butyloxycarbonyl - asparagine p - nitrophenyl ester (1.42 g) in a mixture of dimethylformamide (7 ml) and dichloromethane (21 ml) and shake for 5 hours.

24. Repeat steps 1, 2, 3, 4 and 5.

25. Add a solution of pyroglutamic acid (1.55 g) in dimethylformamide (15 ml) and dichloromethane (41 ml) and shake for 20 minutes.

26. Repeat steps 7 and 1, wash four times with ethanol, filter and dry over KOH.

27. The peptide resin is suspended in trifluoroacetic acid (100 ml) containing mercaptoethanol (1 ml) and anisole (22 ml). Hydrogen bromide is bubbled through while cooling the flask in an ice-water mixture. After 35 minutes, the resin is filtered off and washed twice with trifluoroacetic acid and four times with a mixture of trifluoroacetic acid and dichloromethane (1:1), containing mercaptoethanol and anisole. The combined filtrates are evaporated to dryness and the residue triturated with ether. The solid is filtered and dried (2.5 g).

28. The dinitrophenyl protecting group is removed by treatment with mercaptoethanol at pH 8, and the free peptide is purified by countercurrent distribution and ion-exchange chromatography.

Example 2

Pyroglutamyl - seryl - tryptophyl - prolyl - glycyl - prolyl - asparaginyl - isoleucyl - proline

The title compound is prepared as described for the compound of Example 1, except introducing asparagine in steps 11 and 12 according to the procedures of steps 22 and 23 in place of glutamine and using t-butyloxycarbonyl glycin in place of N^{α}-t-butyloxycarbonyl - N^{Im} dinitrophenyl histidine in step 16, and using t - butyloxycarbonyl - O - benzyl - serine and dicyclohexylcarbodiimide to introduce serine in lieu of steps 22 and 23.

Example 3

Pyroglutamyl - seryl - tryptophyl - prolyl - histidyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared as described for the compound of Example 1, except using tert. - butyloxycarbonyl - O - benzyl - serine and dicyclohexylcarbodiimide to introduce the seryl residue in lieu of steps 22 and 23.

Example 4

Pyroglutamyl - norleucyl - tryptophyl - prolyl - histidyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared as described for the compound of Example 1, except using t - butyloxycarbonyl - norleucine and dicyclohexylcarbodiimide to introduce the norleucyl residue in lieu of steps 22 and 23.

Example 5

Pyroglutamyl - asparaginyl - tryptophyl - prolyl - arginyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared as described for the compound of Example 1, except using t - butoxycarbonyl - nitroarginine for the introduction of the arginyl residue in step 16 in place of N^{α} - t - butoxycarbonyl - N^{Im} dinitrophenyl histidine. The nitro protecting group of the nitro - arginine residue is removed by hydrogenolysis at the end of the synthesis.

Example 6

Pyroglutamyl - seryl - trytophyl - prolyl lysyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared as described for the compound of Example 3, except using N^{α} - t - butyloxycarbonyl - N^{ϵ} - trifluoroacetyl - lysin for the introduction of the lysyl residue in step 16 in place of N^{ϵ} - t -

butyloxycarbonyl - N^{Im} dinitrophenyl histidine. The trifluoroacetyl protecting group is removed by treatment with piperidine at the end of the synthesis.

Example 7

Pyroglutamyl - asparaginyl - tryptophyl - prolyl - histidyl - proline

Starting from t - butyloxycarbonyl - prolyl resin, the title compound is prepared by following the procedure of Example 1 from step 15 to step 27 inclusive.

Example 8

Pyroglutamyl - seryl - tryptophyl - prolyl - histidyl - proline

The title compound is prepared as described for the compound of Example 7 except using t - butyloxycarbonyl - O - benzyl serine and dicyclohexylcarbodiimide to introduce the seryl residue in lieu of steps 22 and 23.

Example 9

Pyroglutamyl - seryl - tryptophyl - prolyl - arginyl - proline

The title compound is prepared as described for the compound of Example 8, except using t-butyloxycarbonyl-nitroarginine to introduce the arginyl residue in step 16 in place of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl histidine. The nitro protecting group is removed by hydrogenolysis at the end of the synthesis.

Example 10

Pyroglutamyl - norleucyl - tryptophyl - prolyl - lysyl - proline

The title compound is prepared as described for the compound of Example 7, except using N^{α} - t - butyloxycarbonyl - N^{ϵ} - trifluoroacetyl lysine to introduce the lysyl residue in step 16 in place of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl histidine, and using t - butyloxycarbonyl - norleucine to introduce the norleucyl residue in lieu of steps 22 and 23. The trifluoroacetyl group is removed by treatment with piperidine at the end of the synthesis.

Example 11

Pyroglutamyl - seryl - tryptophyl - prolyl - glycyl - proline

The title compound is prepared as described for the compound of Example 8, except using t - butyloxycarbonyl glycine to introduce the glycine residue in step 16 in place of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl histidine.

Example 12

Tryptophyl - prolyl - arginyl - proline

Starting from t-butyloxycarbonyl prolyl resin, the procedure of Example 1 is followed from steps 16 to 21, except using t-butyloxycarbonyl-nitroarginine for the introduction of the arginyl residue in step 16 in place of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl histidine. The tetrapeptide is removed from the resin as described in step 27 of Example 1 and the nitro group is then removed by hydrogenolysis.

Example 13

t - Butyloxycarbonyl - tryptophyl - prolyl - nitroarginyl - proline

The nitroarginyl tetrapeptide of Example 12 is acylated with t-butyloxycarbonyl azide at pH 9.

Example 14

Tryptophyl-prolyl-lysyl-proline

The title compound is prepared as described for the compound of Example 12, except using N^{α} - t - butoxycarbonyl - N^{ϵ} - trifluoroacetyl lysine to introduce the lysyl residue in step 16 in place of t - butyloxycarbonylnitroarginine. The trifluoroacetyl group is removed by treatment with piperidine at the end of the synthesis.

Example 15

Tryptophyl-prolyl-histidyl-proline

The title compound is prepared as described for the compound of Example 12 except that step 16 is that described in Example 1.

Example 16

Tryptophyl-prolyl-glycyl-proline

The title compound is prepared as described for the compound of Example 11 but proceeding only as far as step 21.

Example 17

Pyroglutamyl - tryptophyl - prolyl - arginyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared as described for the compound of Example 1 up to step 21, except using t - butyloxycarbonyl - nitroarginine in step 16 in place of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl histidine. After step 21, the preparation continues in step 25 with the incorporation of pyroglutamic acid. The removal of the nitro group from the nitro nonapeptide is achieved by hydrogenolysis at the end of the synthesis.

Example 18

Pyroglutamyl - tryptophyl - prolyl - arginyl - proline

Starting from t - butyloxycarbonyl - prolyl resin the procedure of Example 9 is used. After step 21 the preparation continues with step 25. The pentapeptide is removed from the resin as described in Example 1 (step 27) and the nitro group is removed by hydrogenolysis at the end of the synthesis.

Example 19

Pyroglutamyl - asparaginyl - tryptophyl - prolyl - arginyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared as described for the compound of Example 5 except substituting the procedure of steps 22 and 23 for steps 11 and 12 to introduce the asparaginyl residue in lieu of the glutaminyl residue in step 11.

Example 20

Pyroglutamyl - seryl - tryptophyl - prolyl - arginyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared as described for the compound of Example 2 except using t - butyloxycarbonylnitroarginine to introduce the arginyl residue in step 16 in lieu of t - butoxycarbonylglycine. The nitro protecting group of the nitroarginine residue is removed by hydrogenolysis at the end of the synthesis.

Example 21

Pyroglutamyl - norleucyl - tryptophyl - prolyl - arginyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared as described for the compound of Example 20, except using t - butoxycarbonyl - norleucyl and dicyclohexylcarbodiimide to introduce the norleucyl residue in lieu of steps 22 and 23.

Example 22

Pyroglutamyl - tryptophyl - prolyl - arginyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared as described for the compound of Example 17, except substituting for steps 11 and 12 the procedures of steps 22 and 23 so as to introduce the asparaginyl residue in lieu of the glutaminyl residue.

Example 23

Pyroglutamyl - asparaginyl - tryptophyl - prolyl - histidyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 1 except introducing asparagine in place of glutamine in steps 11 and 12 according to the procedures of steps 22 and 23.

Example 24

Pyroglutamyl - seryl - tryptophyl - prolyl - histidyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 2, except that step 16 is that described in Example 1.

Example 25

Pyroglutamyl - norleucyl - tryptophyl - prolyl - histidyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 4 except introducing asparagine in steps 11 and 12 in place of glutamine according to the procedures of steps 22 and 23.

Example 26

Pyroglutamyl - tryptophyl - prolyl - histidyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 22, except that step 16 is as described in Example 1.

Example 27

Pyroglutamyl - asparaginyl - tryptophyl - prolyl - lysyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 23, except using N^{α} - t - butyloxycarbonyl - N^{ϵ} - trifluoroacetyl - lysine for the introduction of lysine in step 16 in place of N^{α}-t-butyloxycarbonyl-N^{Im} dinitrophenyl histidine. The trifluoroacetyl group is removed by treatment with piperidine at the end of the synthesis.

Example 28

Pyroglutamyl - seryl - tryptophyl - prolyl-lysyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 6, except introducing asparagine in steps 11 and 12 according to the procedure of steps 22 and 23 in place of glutamine.

Example 29

Pyroglutamyl - norleucyl - tryptophyl - prolyl - lysyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 27, except using t - butyloxycarbonylnorleucine and dicyclohexylcarbodiimide to introduce the norleucyl residue in lieu of steps 22 and 23.

Example 30

Pyroglutamyl - tryptophyl - prolyl - lysyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 17, except using N^{α} - t - butyloxycarbonyl - N^{ϵ} - trifluoroacetyl - lysine for the introduction of lysine in step 16 in place of t - butyloxycarbonyl - nitroarginine. The trifluoroacetyl group is removed by treatment with piperidine at the end of the synthesis.

Example 31

Pyroglutamyl - asparaginyl - tryptophyl - prolyl - glycyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 2, except that steps 22 and 23 are those described in Example 1.

Example 32

Pyroglutamyl - norleucyl - tryptophyl - glycyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 2, except using t-butyloxycarbonylnorleucine and dicyclohexylcarbodiimide to introduce the norleucyl residue in lieu of t - butoxycarbonyl - O - benzyl serine and dicyclohexylcarbodiimide.

Example 33

Pyroglutamyl - tryptophyl - prolyl - glycyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 30, except using t - butyloxycarbonyl glycine in step 16 in place of N^{α} - t - butyloxycarbonyl - N^{ϵ} - trifluoroacetyl - lysine.

Example 34

Pyroglutamyl - seryl - tryptophyl - prolyl - arginyl - prolyl - glutaminyl - isoleucyl - proline

The title compound is prepared following the procedure of Example 3, except using t - butyloxycarbonyl - nitroarginine for the introduction of the arginyl residue in step 16 in lieu of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl histidine. The nitro protecting group of the nitroarginine residue is removed by hydrogenolysis at the end of the synthesis.

Example 35

Pyroglutamyl - norleucyl - tryptophyl - prolyl - arginyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 4, except using t - butyloxycarbonyl - nitroarginine for the introduction of the arginyl residue in step 16 in lieu of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl histidine. The nitro protecting group of the nitroarginine residue is removed by hydrogenolysis at the end of the synthesis.

Example 36

Pyroglutamyl - tryptophyl - prolyl - histidyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 17, except that step 16 is that described in Example 1.

Example 37

Pyroglutamyl - asparaginyl - tryptophyl - prolyl - lysyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 1, except using N^{α} - t - butyloxycarbonyl - N^{ϵ} - trifluoroacetyl - lysine for the introduction of the lysyl residue in step 16 in place of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenylhistidine. The trifluoroacetyl group is removed by treatment with piperidine at the end of the synthesis.

Example 38

Pyroglutamyl - norleucyl - tryptophyl - prolyl - lysyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 4, except using N^{α} - t - butyloxycarbonyl - N^{ϵ} - trifluoroacetyl - lysine for the introduction of the lysyl residue in step 16 in place of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl - histidine. The trifluoroacetyl group is removed by treatment with piperidine at the end of the synthesis.

Example 39

Pyroglutamyl - tryptophyl - prolyl - lysyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 17, except using N^{α} - t - butyloxycarbonyl - N^{ϵ} - trifluoroacetyl - lysine for the introduction of the lysyl residue in step 16 in place of t - butyloxycarbonyl - nitroarginine. The trifluoroacetyl group is removed by treatment with piperidine at the end of the synthesis.

Example 40

Pyroglutamyl - asparaginyl - tryptophyl - prolyl - glycyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 5, except using t - butyloxycarbonyl glycine in step 16 in place of t - butyloxycarbinyl - nitroarginine.

Example 41

Pyroglutamyl - seryl - tryptophyl - prolyl - glycyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 3, except using t - butyloxycarbonyl glycine in step 16 in lieu of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl histidine.

Example 42

Pyroglutamyl - norleucyl - tryptophyl - prolyl - glycyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared following

the procedure of Example 4, except using t - butyloxycarbonyl glycine in lieu of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl histidine.

Example 43

Pyroglutamyl - tryptophyl - prolyl - glycyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 17, except using t - butyloxycarbonyl glycine in step 16 in place of t - butyloxycarbonyl - nitroarginine.

Example 44

Pyroglutamyl - asparaginyl - tryptophyl - prolyl - arginyl - proline

The title compound is prepared following the procedure of Example 7, except using t - butyloxycarbonyl - nitroarginine for the introduction of the arginyl residue in step 16 in place of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl histidine. The nitro protecting group of the nitroarginine residue is removed by hydrogenolysis at the end of the synthesis.

Example 45

Pyroglutamyl - norleucyl - trytophyl - prolyl - arginyl - proline

The title compound is prepared following the procedure of Example 10, except using t - butyloxycarbonyl - nitroarginine for the introduction of the arginyl residue in step 16 in place of N^{α} - t - butyloxycarbonyl - N^{ϵ} - trifluoroacetyl lysine. The nitro protecting group of the nitroarginine residue is removed by hydrogenolysis at the end of the synthesis.

Example 46

Pyroglutamyl - norleucyl - tryptophyl - proline - histidyl - proline

The title compound is prepared following the procedure of Example 7, except using t - butyloxycarbonyl - norleucine and dicyclohexylcarbodiimide to introduce the norleucyl residue in lieu of steps 22 and 23.

Example 47

Pyroglutamyl - tryptophyl - prolyl - histidyl - proline

The title compound is prepared following the procedure of Example 18, except that step 16 is that described in Example 1.

Example 48

Pyroglutamyl - asparaginyl - tryptophyl - prolyl - lysyl - proline

The title compound is prepared following the procedure of Example 7, except using N^{α} - t - butyloxycarbonyl - N^{ϵ} - trifluoroacetyl - lysine for the introduction of the lysyl residue in step 16 in place of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl histidine. The trifluoroacetyl group is removed by treatment with piperidine at the end of the synthesis.

Example 49

Pyroglutamyl - seryl - trytophyl - prolyl - lysyl - proline

The title compound is prepared following the procedure of Example 8, except using N^{α} - t - butyloxycarbonyl - N^{ϵ} - trifluoroacetyl - lysine for the introduction of the lysyl residue in step 16 in place of N^{α} - t - butyloxycarbinyl - N^{Im} dinitrophenyl histidine. The trifluoroacetyl group is removed by treatment with piperidine at the end of the synthesis.

Example 50

Pyroglutamyl - tryptophyl - prolyl - lysyl - proline

The title compound is prepared following the procedure of Example 18, except using N^{α} - t - butyloxycarbonyl - N^{ϵ} - trifluoroacetyl - lysine for the introduction of the lysyl residue in step 16 in place of t - butyloxycarbonyl - nitroargine. The trifluoroacetyl group is removed by treatment with piperidine at the end of the synthesis.

Example 51

Pyroglutamyl - asparaginyl - tryptophyl - prolyl - glycyl - proline

The title compound is prepared following the procedure of Example 7, except using t - butyloxycarbonyl glycine in step 16 in lieu of N^{α} - t - butyloxycarbonyl - N^{Im} dinitrophenyl histidine.

Example 52

Pyroglutamyl - norleucyl - tryptophyl - prolyl - glycyl - proline

The title compound is prepared following the procedure of Example 10, except using t - butyloxycarbonyl glycine in step 16 in place of N^{α} - t - butyloxycarbonyl - N^{ϵ} - trifluoroacetyl lysine.

Example 53

Pyroglutamyl - trytophyl - prolyl - glycyl - proline

The title compound is prepared following the procedure of Example 18, except using t - butyloxycarbonyl glycine in step 16 in place of t - butyloxycarbonyl - nitroarginine.

Example 54

Asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 2 from steps 1 to 12 inclusive. The protected peptide is then removed from the resin according to step 27. The t - butyloxycarbonyl protecting group is removed during this step.

Example 55

Glutaminyl-isoleucyl-prolyl-proline

The title compound is prepared following the procedure of Example 1 from steps 1 to 12 inclusive. The protected peptide is then

removed from the resin according to step 27. The t-butyloxycarbonyl protecting group is removed during this step.

Example 56

Prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 2 from steps 1 to 14 inclusive. The protected peptide is then removed from the resin according to step 27. The t-butyloxycarbonyl protecting group is removed during this step.

Example 57

Prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 1 from steps 1 to 14 inclusive. The protected peptide is then removed from the resin according to step 27. The t - butyloxycarbonyl protecting group is removed during this step.

Example 58

Histidyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 23 from steps 1 to 16 inclusive. The protected peptide is then removed from the resin according to step 27. The t-butyloxycarbonyl protecting group is removed during this step.

Example 59

Arginyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 19 from steps 1 to 16 inclusive. The protected peptide is then removed from the resin according to step 27. The t-butyloxycarbonyl protecting group is removed during this step.

Example 60

Lysyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 27 from steps 1 to 16 inclusive. The protected peptide is then removed from the resin according to step 27. The t-butyloxycarbonyl protecting group is removed during this step.

Example 61

Glycyl - prolyl - asparaginyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 2 from steps 1 to 16 inclusive. The protected peptide is then removed from the resin according to step 27. The t - butyloxycarbonyl protecting group is removed during this step.

Example 62

Arginyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 5 from steps 1 to 16 inclusive. The protected peptide is then removed from the resin according to step 27. The t-butyloxycarbonyl protecting group is removed during this step.

Example 63

Histidyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 1 from steps 1 to 16 inclusive. The protected peptide is then removed from the resin according to step 27. The t-butyloxycarbonyl protecting group is removed during this step.

Example 64

Glycyl - prolyl - glutaminyl - isoleucyl - prolyl - proline

The title compound is prepared following the procedure of Example 40 from steps 1 to 16 inclusive. The protected peptide is then removed from the resin according to step 27. The t-butyloxycarbonyl protecting group is removed during this step.

Example 65

t - Butyloxycarbonyl - tryptophyl - prolyl - arginyl - proline

The tetrapeptide of Example 12 is acylated with t-butyloxycarbonyl azide at pH 9 to yield the title compound.

Example 66

N^{α} - t - Butyloxycarbonyl - tryptophyl - prolyl - N^{ϵ} - t - butyloxycarbonyl - lysyl - proline

The tetrapeptide of Example 14 is acylated with t-butyloxycarbonyl azide at pH 10 to yield the title compound.

Example 67

t - Butyloxycarbonyl - tryptophyl - prolyl - histidyl - proline

The tetrapeptide of Example 15 is acylated with t-butyloxycarbonyl azide at pH 9 to yield the title compound.

Example 68

t - Butyloxycarbonyl - tryptophyl - prolyl - glycyl - proline

The tetrapeptide of Example 16 is acylated with t-butyloxycarbonyl azide at pH 9 to yield the title compound.

Certain peptides which may be used as intermediates in preparing the peptides of the present invention, and the preparation of these intermediate peptides are described and claimed in our Patent Application No. 21794/73 (Serial No. 1357122), which is a divisional of the present application.

WHAT WE CLAIM IS:—

1. A peptide of the general formula

Pyroglutamyl-$(X)_n$-tryptophyl-prolyl-Y-prolyl-Z-isoleucyl-prolyl-proline,

wherein X is asparaginyl, seryl, or norleucyl, n is 0 or 1, Y is arginyl, histidyl, lysyl or glycyl, and Z is asparaginyl or glutaminyl, or a derivative of said peptide in which at least one carboxyl, amino, guandino or hydroxy group is protected, or a physiologically acceptable acid addition salt of said peptide or derivative thereof.

2. A peptide of the general formula

pyroglutamyl-$(X_n$-trypophyl-prolyl-Y-proline

wherein X, n and Y are as defined in Claim 1, a derivative of said peptide in which at least one carboxyl, amino, guanidino or hydroxy group is protected, or a physiologically acceptable acid addition salt of said peptide or derivative.

3. A peptide of the general formula

tryptophyl-prolyl-Y-proline

wherein Y is as defined in Claim 1, a derivative of said peptide in which the amino group of the tryptophyl radical is protected, a derivative in which, in addition, at least one other group from among amino, carboxyl and guanidino groups is protected, or a physiologically acceptable acid addition salt of any of said derivatives.

4. A tetra peptide or pharmaceutically acceptable salt or a protected derivative thereof, as claimed in any of claims 1 to 3 and substantially as described.

5. A method for inhibiting the conversion of angitensin I into angiotensin II in non-humans, which comprises contacting angiotensin I with from about 0.05 to about 10 mcg/ml of a compound which is:—

I. A nona- or decapeptide of the general formula

Pry-$(X)_n$-Trp-Pro-Y-Pro-Z-Ile-Pro-Pro,

or

II. A penta- or hexapeptide of the general formula

Pyr-$(X)_n$-Trp-Pro-Y-Pro,

or

III. A tetrapeptide of the general formula

Trp-Pro-Y-Pro,

in which the terminal amino group is protected, or a physiologically acceptable acid addition salt thereof, or a derivative in which at least one carboxy, amino, guanidino or hydroxy group is protected, in addition to the terminal amino group, X, Y, Z and n being as defined in Claim 1.

6. A process for preparing a peptide of the formula

R^1-Pro-Y-Pro-B

wherein:

Y is an arginine, histidine, lysine or glycine moiety,

B is hydroxy or —Z-Ile-Pro-Pro wherein Z is an asparagine or glutamine moiety, and

R^1 is a tryptophyl radical in which the amino group is protected, or

Pyr-$(X)_n$-Trp

when B is hydroxy, or Pyr-$(X)_n$- when when B is —Z-Ile-Pro-Pro, X is an asparagine, serine, or norleucine moiety and n is zero or 1,

which comprises converting the amino group of the tryptophyl radical in a peptide of the formula

Trp-Pro-Y-Pro-OH

into a protected amino group or introducing to the Trp-terminal portion of a peptide of formula

Trp-Pro-Y-Pro-OH

or

Trp-Pro-Y-Pro-Z-Ile-Pro-Proline

the group $(X)_n$ and then a pyroglutamyl group.

7. A compound as claimed in any of claims 1 to 3 substantially as described in any of the Examples.

8. A process for preparing a compound as claimed in any of claims 1 to 4 and 7, substantially as described in any of the Examples.

9. A method as claimed in claim 5 for inhibiting the conversion of angiotensin I into angiotensin II in non-humans, substantially as herein described.

Agents for the Applicant,
STANLEY, POPPLEWELL, FRANCIS & ROSS,
Chartered Patent Agents,
20/21 Tooks Court,
Cursitor Street,
London, EC4A 1LB.

Printed for Her Majesty's Stationery Office, by the Courier Press, Leamington Spa, 1974.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.